REMARKS

INTRODUCTORY STATEMENT:

The present application was filed on March 28, 2001, with 114 claims. On July 11, 2002, the Office mailed a Restriction Requirement and Species Election for which the claims of Group I, claims 1-39, were elected with traverse. In the response to the Restriction Requirement, applicants requested that the Examiner regroup the claims so that the claims of Group I, II, and III form one group and the claims of Groups IV, V, and VI form another group.

In the Office Action under reply, the Examiner regrouped the claims of Groups IV, V, and VI into Group IV but refused to regroup the claims of Groups I, II, and III into one group. Accordingly, claims 1-39 were examined and were subjected to the following actions: claims 1, 14, 20, and 21 were objected to on informal grounds and claims 1, 2, 5-15, 27-31, and 34-39 were rejected under 35 U.S.C. § 112, second paragraph.

With the present Amendment, claims 40-114 have been canceled without prejudice or disclaimer and applicants reserve the right to file one or more divisional applications on the canceled claims; claims 1-2, 5-7, 14, 17, 18, 20, 22-24, 34, 37, and 38 have been amended as explained below; and minor amendments have been made to the specification to correct inadvertent errors in grammar and syntax and to add commas and articles where appropriate. In addition to the foregoing, claims 3 and 4 have been amended to more clearly define the invention and not for purposes of patentability. For the Examiner's convenience, a copy of the pending claims as they will appear after entry of the foregoing amendments is attached as Appendix C.

CLAIM OBJECTIONS:

Claims 1, 14, and 20 were objected to on informal grammatical grounds and claim 21 was objected to as being in improper dependent form for failing to further limit the subject matter of a previous claim.

Claims 1 and 14 have been amended in accordance with the Examiner's suggestions; accordingly, the objections to these claims are now moot.

With respect to claim 20, this objection is respectfully traversed. The Examiner suggests changing the language of the claims from "an RNA" to "a RNA." While the article "a" is usually used to identify a noun starting with a consonant and the article "an" is usually used to identify a noun starting with a vowel, when an acronym starting with a consonant *sounds* like it starts with a vowel, the article "an" is used instead of the article "a." As support, please find attached the website page from an on-line grammar database that supports this grammatical principle. Based upon the foregoing, applicants

respectfully request that the Examiner withdraw his objection to claim 20 and allow this claim to stand with the language "an RNA."

With respect to claim 21, this objection is also respectfully traversed. Because the invention may be used for *inter alia* DNA amplification as well as genetic analysis (see specification, page 6, lines 14-15), claim 21 further limits the invention by reciting that its use pertains to genetic analysis. Accordingly, applicants respectfully request reconsideration and withdrawal of this objection.

CLAIM REJECTIONS - 35 U.S.C. § 112

Claims 1, 2, 5-15, 18-25, 27-31, and 34-39 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 2 were rejected on the grounds that the language "each oligonucleotide probe comprising a sequence segment complementary, or complementary except at a position corresponding to position of the target sequence in each oligonucleotide probe" is vague. The Examiner suggests that the language "a position corresponding to a position of the target sequence in each oligonucleotide probe" means a possible mismatch region in the target sequence segment after the formation of a hybridization complex between each oligonucleotide probe and the target sequence segment. For the reasons set forth below, the Examiner's proposed explanation is not correct.

First, applicants respectfully note that the identified language in claims 1 and 2 is actually "...each oligonucleotide probe comprising a sequence segment complementary, or complementary except at a position corresponding to a *probed position* of the target sequence..." (emphasis added here). The "probed position," also referred to as the "position of interest," is identified on page 6 of the specification as the position of the target sequence segment pairing to a degenerately pairing nucleotide (lines 21-23). For purposes of clarity and not for purposes of patentability, the phrase "probed position" was changed to a "position of interest." In addition to the foregoing, the claim language was clarified to recite "...a sequence segment complementary to the target sequence segment, or complementary to the target sequence segment except at a position corresponding to a position of interest..."

Second, with respect to the Examiner's suggested interpretation of the claim language, it is noted that in the last wherein clause of claim 1, it is recited that hybridization of each oligonucleotide probe to the target sequence segment occurs only if no mismatch exists at the position of interest. Because hybridization will not occur in the event of a mismatch at the position of interest, it follows that the language identified by the Examiner, i.e., the clause of claim 1 reciting the contact of the oligonucleotides

with the target sequence segment under hybridizing conditions, will not be successful if a mismatch exists at the position of interest. See also, specification, page 18, line 14.

In addition to the foregoing, both claims 1 and 2 were also rejected for the use of the language "may." Both of these claims have been rewritten and the word "may" has been replaced with the word "are."

Claim 5 was rejected on the grounds that the following language lacks sufficient antecedent basis: "null hybridizing sequence" and "nucleic acid sequence of interest." With respect to the "null hybridizing sequence," claim 5 has been amended to positively recite the "null hybridizing sequence" as an additional claim limitation. With respect to the "nucleic acid sequence of interest," this language has been changed to "target sequence segment," which is clearly and properly recited in claim 1.

Claim 6 was rejected on the grounds that the recited "sequencing method" lacks antecedent basis. This language has been amended to recite that the method is used to sequence the target nucleic acid analyte.

The Examiner rejected claim 7 (misidentified as claim 5 in the Office Action) on the grounds that the language "an array of oligonucleotide probes" lacks sufficient antecedent basis. Claim 7 has been amended to positively recite an array of oligonucleotide probes as an additional claim limitation.

Claim 14 was rejected on the grounds that the language "a label intrinsic to the target sequence segment" is unclear. This language has been replaced with the "target signal" language suggested by the Examiner.

Claim 18 was rejected as unclear. This claim has been amended to recite that the hybridized target nucleic acids are amplified by a polymerase enzyme that requires a hybridized complex for amplifying a nucleic acid sequence.

Claim 20 was rejected as lacking sufficient antecedent basis. Claim 20 has been amended to depend from claim 18, which recites the use of a polymerase enzyme, rather than claim 19, which recites the use of a DNA polymerase.

Claim 23 was rejected as lacking insufficient antecedent basis and claim 24 was rejected as unclear. Claim 23 has been amended to recite that the target nucleic acid analyte is derived from genomic DNA and claim 24 has been amended to recite that the target nucleic acid analyte is derived from a cDNA.

Claim 34 was rejected on the grounds that the language "the second label moiety" lacks sufficient antecedent basis. Claim 34 has been amended to depend from claim 33 rather than claim 18, thus providing proper antecedent basis.

Claim 37 was rejected on the grounds that the language "kinetics of hybridization" is unclear. Claim 37 has been amended to remove the reference to kinetics (which is referenced on page 46, line 3, of the specification).

Claim 38 was rejected on the grounds that the language "the electric potential" lacks sufficient antecedent basis. Claim 38 has been amended to depend from claim 39 rather than claim 14, thus providing proper antecedent basis.

In light of the foregoing amendments and remarks, applicants respectfully request reconsideration and withdrawal of all indefiniteness rejections.

CONCLUSION

Because all of the claim objections and claim rejections set forth by the Examiner have been addressed and resolved with this Amendment, applicants respectfully request withdrawal of all claim objections and rejections and early passage of this application to issue.

The Examiner is welcome to contact the undersigned attorney at 650-330-4913 to discuss any issues regarding this Amendment.

Respectfully submitted,

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APPENDIX A

VERSION OF AMENDMENTS TO THE SPECIFICATION WITH MARKINGS TO SHOW CHANGES MADE

Changes to page 6, lines 16-18.

The invention provides a method of reducing the required number of unique hybridizing sequences that may be used to hybridize to a nucleic acid sequence of interest under hybridizing conditions. The method involves hybridizing to the nucleic acid sequence of interest a first hybridizing nucleotide sequence and a second hybridizing nucleotide sequence, each hybridizing nucleotide sequence comprising a sequence segment complementary to a nucleic acid sequence of interest, or complementary to a nucleic acid sequence of interest except at a position of interest or probed position which comprises the position pairing to a degenerately pairing nucleotide~~, to a nucleic acid sequence of interest~~. Additional probes or hybridizing nucleotide sequences are required if there are more than four nucleotides that may be present at the variable position or position of interest. For four possible nucleotides in a sequence, two nucleic acid hybridizing sequences are required each having a nucleotide base pairing to a set of two nucleotides at the variable position, the two sets overlapping in one nucleotide, which is common to both sets.

Changes to page 16, line 15, through page 17, line 2.

The term "hybridization probe" as used herein refers to a nucleic acid sequence that by itself or as a member of a set of nucleic acid sequences or probes for a specific nucleic acid sequence, effects the hybridization of a specific target sequence. The hybridization probes of the invention comprise a nucleic acid sequence segment having sequence complementary to the analyte sequence of interest. Such probes may comprise nucleic acid sequence for potential hybridization with analyte only, or may additionally comprise ~~and~~ a discrete tagging or labeling moiety, such as a chemiluminescent moiety or a discrete nucleic acid sequence that is not a putative anti-target or anti-analyte sequence, but functions solely to indicate the presence of the probe. Such hybridization probes include sequences that form hybrids for enzymatic amplification such as primers for polymerase chain reaction amplification and sequences forming double stranded complex replication templates for enzymes such as the RNA replicases. In addition to hybridizing probes for an amplification process, probes for simple hybridization and detection, both tagged or labeled with a discrete moiety and not labeled with any discrete label moiety are contemplated. Nucleic acid sequences comprising probes not having a discrete labeling moiety may be intrinsically labeled for detection of the hybridization, as by incorporation of ^{32}P into the nucleic acid phosphodiester backbone or the like. Hybridization probes may comprise a sequence complementary to the sequence to be detected and detectable signal or marker indicating the presence of the complementary sequence, for example a separate moiety such

as a chemiluminescent marker, or ^{32}P incorporated into the phosphodiester backbone of the nucleic acid sequence or both.

Changes to page 18, line 32, through page 19, line 13.

The sequence is deduced by reassembly of the sequence of known (N-1)-mer overlapping oligonucleotides that hybridize to the target nucleic acid to generate the sequence of the target nucleic acid, which cannot be accomplished in some cases because some information is lost if the target nucleic acid is not in fragments of appropriate length in relation to the size of the ~~oligonucleotide that is~~oligonucleotides that are used for the hybridization probes. The quantity of information lost is proportional to the length of a target being sequenced. However, if sufficiently short targets are employed, their sequence can be unambiguously determined. The deductive construction of the sequence is interrupted in analyte sequence regions where a given overlapping (N-1)-mer is duplicated to appear at least three times in succession, e.g. repeated two or more times, causing the deduced sequence to skip the second and subsequent repetitions in sequence. At such points either of two different N-mers, differing in the last nucleotide are deduced for extending the sequence construction. Such branching points of sequence deduction limit unambiguous assembly of a sequence.

Changes to page 20, lines 23-33.

Another sequencing method that relies upon hybridization employs a label or tag that identifies the specific hybridizing sequence. For example a different fluorescent marker can be linked to each possible sequence of three nucleotides (4^3 or 64 in all), and a sequence may be obtained by successive hybridization and digestion of three nucleotides at a time. The sequence may also be obtained by labels comprising a nucleotide sequence, for example the start codon AUG may be labeled by the sequence 5'-AAAAAAAAACCCCCTTTTCTTTT (SEQ ID NO: 11), which will form a hairpin loop self complementary structure that can be differentiated from like labeling structures, such as 5'-AAAAAAAAACCCCCTTTTTTTTT (SEQ ID NO: 12) and 5'-AAAAGAAAACCCCCTTTTCTTTT (SEQ ID NO: 13), by the temperature that causes a loss of such secondary structure.

Changes to page 22, line 23, to page 23, line 29.

The nucleic acid analog 8-oxo-dGTP (Amersham, Cambridge UK) is formed spontaneously by oxidation of dGTP in the context of normal cellular metabolic activity. 8-oxo-dGTP has one form which can behave as either dG to pair with C (FIG. 2) in a standard base pairing steric arrangement or as dT to pair with A (FIG. 3) in a sterically atypical base pairing arrangement resembling a wobble base pairing arrangement. Thus 8-oxo-dG at a position in a nucleic acid sequence pairs with both C (as dG) and A (as dT) in close amounts indicative of moderately different binding energies. Thus 8-oxo-dG may be incorporated in a nucleic acid sequence for either G or T almost equally in a proportion relative to the total number of G or T in the polymerization mixture. When replicated a position incorporating 8-oxo-dG is polymerized as the complementary sequence to the template having 8-oxo-dG incorporated at the position of interest as a G, therefore causing only dC to be incorporated at that incoming nucleotide position because of the difference in free energies between the two base pairing interactions, e.g. 8-oxo-dG::C versus 8-oxo-dG::A. FIG 2 shows that 8-oxo-dG::C has three H bonding interactions compared to two for 8-oxo-dG::A (FIG. 3), which is not a standard Watson-Crick base pairing interaction. Because in a polymerase reaction mixture containing all the nucleotides plus 8-oxo-dG, a proportion of sequence positions having a T (pairing A) are substituted with 8-oxo-dG, which then pairs with C, the purine A is effectively converted to the pyrimidine C, and T is converted to G. Such random or stochastic transmutation is from purine to pyrimidine and visa versa, a transmutation termed transversion. Note that dGTP could be absent from the polymerase mixture and wholly replaced by 8-oxo-dG, but this will not typically be the case. Because dTTP and 8-oxo-dGTP are necessarily present in the reaction mixture, the replacement of T with 8-oxo-dG will be proportionate to the relative amounts, and therefore concentrations of the two dNTPs. For replication, the presence of the 8-oxo-dG causes the incoming nucleotide for the complementary nascent strand synthesized from the 8-oxo-dG containing template to be dC exclusively, and the dC then causes a dG to be inserted for subsequent polymerization using the new strand as a template. Thus, the 8-oxo-dG in a sequence behaves as a G for the purpose of synthesis from a template containing the 8-oxo-dG. If all four standard dNTPs (A,T,C,G) are present in the polymerization mixture along with 8-oxo-dG, then ~~because~~ the sequence having random substitution of 8-oxo-dG for T forms a template for further polymerization in which the complementary substitution of A for C occurs along with the complementary substitution of T for G. Such mutations from purine to pyrimidine and visa versa are known as transversion mutations. Thus although mechanistically somewhat different than the random mutagenesis effected via dPTP, while still depending upon degenerate base pairing, 8-oxo-dG is used as a nucleotide substrate of the polymerase in conjunction with PCR to randomly or stochastically interchange T and G and consequently complementary interchange A and C. This is therefore a PCR mediated random transversion mutagenesis, converting T to G and A to C, but ~~no~~ not the converse (e.g., neither G to T nor C to A).

APPENDIX B

VERSION OF CLAIMS AMENDMENTS WITH MARKINGS TO SHOW CHANGES MADE

Changes to claims 1-7, 14, 17, 18, 20, 22-24, 34, 37, and 38.

1. **(Amended)** A method of employing oligonucleotide probes to obtain information on a target nucleic acid analyte containing a target sequence segment, the method comprising:

contacting the analyte, under hybridizing conditions, with at least two oligonucleotide probes, each oligonucleotide probe comprising a sequence segment complementary~~, or complementary except at a position corresponding to a probed position of~~ to the target sequence or complementary to the target sequence except at a position of interest,

~~wherein a nucleotide at the position of each oligonucleotide probe corresponding to the probed position is capable base pairing with a set of two or more nucleotides, each set is unique but includes one nucleotide common to all the sets, and one nucleotide that may be present in the target sequence segment is not represented in any set,~~wherein each of the at least two oligonucleotide probes has one nucleotide capable of base pairing with a set of two or more nucleotides, said set of two or more nucleotides including one nucleotide common to all sets and lacking one nucleotide present in the target sequence segment; and

~~further~~ wherein hybridization of each oligonucleotide probe to the target sequence segment under the hybridizing conditions occurs only if no mismatch exists at the ~~probed~~ position of interest, such that depending upon the identity of the nucleotide at the ~~probed~~ position of interest ~~of the target sequence segment~~, all, some or none of the at least two oligonucleotide probes hybridize to the target ~~nucleic acid~~ sequence segment.

2. **(Amended)** The method of claim 1 wherein the target sequence segment of the analyte has four nucleotides ~~may be present in the target sequence segment and each oligonucleotide probe comprises~~and at least two oligonucleotide probes are used, each oligonucleotide probe comprising, at ~~the~~ a position corresponding to the ~~probed~~ position of interest, a nucleotide base pairing with two of the four nucleotides present in the target sequence segment.

3. **(Amended)** The method of claim 1 wherein ~~more than four~~ the target sequence segment of the analyte has at least five nucleotides ~~may be present in the target sequence segment, each oligonucleotide probe comprises~~ and at least three oligonucleotide probes are used, each oligonucleotide probe comprising, at ~~the~~ a position corresponding to the ~~probed~~ position of interest, a nucleotide base

pairing with ~~more than two~~at least three of the at least five nucleotides present in the target sequence segment ~~and more than two oligonucleotide probes are employed~~.

4. **(Amended)** The method of claim 3 wherein the target sequence segment of the analyte has five nucleotides ~~may be present in the target sequence segment,~~ and three oligonucleotide probes are ~~employed~~used, each oligonucleotide probe ~~comprises,~~ comprising at ~~the~~ a position corresponding to ~~the~~ a variable position of the target sequence segment, a nucleotide base pairing with a set of two or three nucleotides, ~~and each set has at least one nucleotide not common to all the sets in common with another set~~ wherein the sets having nucleic acid sequences in common each lack at least one nucleotide not common to the sets.

5. **(Amended)** The method of claim 1 ~~wherein~~ further comprising a null hybridizing sequence comprising a nucleic acid sequence, complementary to the ~~nucleic acid sequence of interest~~ target sequence segment,

wherein the null hybridizing sequence is base paired with a set of two or more nucleotides at a variable position of the target sequence segment; and

wherein ~~having the~~a nucleotide represented in neither ~~the~~ a first or second set of the two or more nucleotides at the variable position is ~~employed~~used to probe the target sequence segment.

6. **(Amended)** The method of claim 1 ~~employed as a sequencing method~~used for sequencing the target nucleic acid analyte.

7. **(Amended)** The method of claim 6, further comprising an array of oligonucleotide probes, wherein the ~~sequencing method~~ sequence of the target nucleic acid analyte is determined by analysis of hybridization data obtained from ~~an~~ the array of oligonucleotide probes.

14. **(Amended)** The method of claim 9 wherein detection of a target sequence segment hybridizing to an oligonucleotide probe is by detection of ~~an label intrinsic to the target sequence segment~~a target signal.

17. **(Amended)** The method of claim 6 wherein the sequencing method is by detection of labels that attach ~~to~~ by hybridization to the target sequence segment.

18. **(Amended)** The method of claim 1 wherein hybridized target nucleic acids are amplified by a polymerase enzyme that requires a hybridized complex for ~~polymerizing the formation of~~ amplifying a nucleic acid sequence.

20. **(Amended)** The method of claim ~~19~~ 18 wherein hybridized nucleic acids are amplified by an RNA replicase enzyme.

22. **(Amended)** The method of claim ~~21~~ 1 ~~employed~~ used for allelic analysis.

23. **(Amended)** The method of claim ~~21~~ 1 wherein the target nucleic acid analyte is derived from genomic DNA ~~is analyzed~~.

24. **(Amended)** The method of claim ~~21~~ 1 wherein ~~genomic cDNA is analyzed~~ the target nucleic acid analyte is derived from a cDNA.

34. **(Amended)** The method of claim ~~18~~ 33 wherein the second label moiety comprises a luminescent moiety.

37. **(Amended)** The method of claim 36 wherein the ~~enhancement is increasing stringency or kinetics of hybridization~~hybridization is enhanced by increasing hybridization stringency.

38. **(Amended)** The method of claim ~~14~~ 39 wherein the electric potential at the substrate surface is electronically controlled to enhance hybridization.